

# Effects of $\text{Ca}^{2+}$ concentrations on accumulations of mineral elements in the components of *Pteroceltis tatarinowii*

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**Abstract:** The bark of *Pteroceltis tatarinowii* is a raw material for manufacturing Xuan Paper. The effects of  $\text{Ca}^{2+}$  concentrations on the accumulation of mineral elements in the bark, leaf and root of *Pteroceltis tatarinowii* were studied under controlled conditions. The types of Hoagland nutrient solution with three  $\text{Ca}^{2+}$  concentrations levels (200, 400 and 600  $\mu\text{g} \cdot \text{g}^{-1}$ ) and a control (without  $\text{Ca}^{2+}$ ) were designed to culture *Pteroceltis tatarinowii*. After 6 months, contents of seven mineral elements including Ca, K, Mg, Mn, Zn, Cu and Na in the root, leaf and bark were analyzed. The results indicated that Ca accumulations content in the root, leaf and bark had positively relation with  $\text{Ca}^{2+}$  concentrations (200, 400, 600  $\mu\text{g} \cdot \text{g}^{-1}$ ), and the order of the Ca content in the three components was root>leaf>bark. Ca content in the root treated with 600  $\mu\text{g} \cdot \text{g}^{-1}$   $\text{Ca}^{2+}$  concentrations was 5.5 times as high as that of the control, and about 1.4 times as high as that of the root treated in 200 and 400  $\mu\text{g} \cdot \text{g}^{-1}$   $\text{Ca}^{2+}$  concentrations respectively. On the contrary, K and Mg contents in the root, leaf and bark were negatively related to  $\text{Ca}^{2+}$  concentrations, especially in the bark, and their accumulation trend followed the order of leaf>root>bark. K content in the bark treated with 600  $\mu\text{g} \cdot \text{g}^{-1}$   $\text{Ca}^{2+}$  concentrations was 39.3% of that of the control, and was 79.0% and 91.8% of that of the bark treated with 200  $\mu\text{g} \cdot \text{g}^{-1}$  and 400  $\mu\text{g} \cdot \text{g}^{-1}$   $\text{Ca}^{2+}$  concentrations respectively; Mg content in the bark treated with 600  $\mu\text{g} \cdot \text{g}^{-1}$   $\text{Ca}^{2+}$  concentrations was 23.4% of that of the control, and was 27.1% and 35.4% of that of the bark treated with 200 and 400  $\mu\text{g} \cdot \text{g}^{-1}$   $\text{Ca}^{2+}$  concentrations respectively. Compared with the control, the general tendency of Mn, Zn and Cu content decreased with increasing of  $\text{Ca}^{2+}$  concentrations and their contents were in the order: root>leaf>bark. Based on the results of this study, the experiment has been useful for providing academic bases in improving the bark quality of *Pteroceltis tatarinowii* on non-limestone soil.

**Keywords:** *Pteroceltis tatarinowii*; Hoagland nutrient solution;  $\text{Ca}^{2+}$  concentrations; Mineral element; Component

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## Introduction

*Pteroceltis tatarinowii*, a deciduous and a mid-growing tree species, only grows naturally in China and mainly distributes in limestone mountainous region. It is one of the China's third class protective tree species and an indicative plant of limestone soil. The bark of *Pteroceltis tatarinowii* is the main raw materials for manufacturing Xuan paper, which is the famous traditional handwriting paper in China and one of the "Four Treasures of the Study" for traditional Chinese stationery (Cao 1993). Liu *et al.* (1985; 1986) indicated that the bark quality, which was positively affected by the content of  $\text{CaCO}_3$  in its cell wall, had a direct influence on the quality of Xuan paper. Now many papermaking factories like to buy the bark harvested from limestone soil, however there are not enough *Pteroceltis tatarinowii* plantations, which grow on the limestone soil. As a result, it had a great negative effect on production and quality of Xuan paper. To solve these problems, researchers in China have done some studies on seed source selection (Fang 1998),

seedling culture techniques (Fu *et al.* 2001; Yang *et al.* 1996), silvicultural practices (Fang 1996; Jiang *et al.* 1992; Duan *et al.* 1996; Zhan *et al.* 1994). They also studied on the effect of site conditions and management practices on the productivity and the bark quality of *Pteroceltis tatarinowii* (Fang *et al.* 2001; Li *et al.* 2001) and other related techniques (Fu *et al.* 2002; Tao 1989; Liu 1996). Some investigations showed that mineral element contents in the bark of *Pteroceltis tatarinowii* were affected by site conditions; especially the Ca and Mg contents were much higher in the limestone soil (Fang *et al.* 2002). This study was designed to test the effects of  $\text{Ca}^{2+}$  concentrations on the accumulation of mineral elements in the leaf, root and bark of *Pteroceltis tatarinowii* under controlled condition so as to provide some fundamental bases for the silviculture of *Pteroceltis tatarinowii* plantations.

## Materials and methods

### Materials

*Pteroceltis tatarinowii* seeds were collected from Qingyang county of Anhui Province in 2000. Their mother trees were nine years old and grew in limestone soil. Seeds were sowed in containers with vermiculite after they were treated in strata (seeds mixed with sand). When the height of the

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seedlings was about 5 cm, they were transplanted into Hoagland nutrient solution (Shen 1992).

## Methods

### Experiment treatments

The experiment was conducted in the glasshouse of Nanjing Forestry University from February to November in 2001. In this experiment there were three treatment levels, i.e. three  $\text{Ca}^{2+}$  concentrations (200, 400; 600  $\text{g kg}^{-1}$ ) and a control (without  $\text{Ca}^{2+}$ ), and three replications. The  $\text{Ca}^{2+}$  concentrations in the Hoagland nutrient solution were regulated by adding calcium nitrate. During the experiment, the solution had been aerated every day and renewed each week since the seedlings were transplanted into it.

### Measurement, sampling and analysis

After ten days when the seedlings were transplanted into the solutions, base diameter and seedling height were measured every ten days. In November, samples of the leaf, root and bark of *Pteroceltis tatarinowii* seedlings were collected for mineral element analysis. Individual seedling samples were bulked into per treatment, oven-dried (65 °C), then mixed, weighed, ground, finely sieved. Ca, K, Mg, Mn, Zn, Cu and Na were determined by Atomic Absorption Spectroscopy (Issac *et al.* 1971; Nanjing Agriculture University 1986). The biomass production for each treatment was also determined based on mean base diameter and height.

## Results

### Effects of $\text{Ca}^{2+}$ concentrations on Ca content in the root, leaf and bark

Ca contents in the root, leaf and bark of the control (without  $\text{Ca}^{2+}$  in the solution) had no significant difference, from 6.3  $\text{g kg}^{-1}$  to 7.6  $\text{g kg}^{-1}$ , while the difference of  $\text{Ca}^{2+}$  content in the three components in treatments was very significant (Fig.1). Both variance analysis and Duncan's multiple range tests indicated that the differences of Ca content in root treated with 600  $\mu\text{g g}^{-1}$   $\text{Ca}^{2+}$  concentrations and the others were very significant ( $F=6.48$ ), but were not significant in 200  $\mu\text{g g}^{-1}$  and 400  $\mu\text{g g}^{-1}$   $\text{Ca}^{2+}$  concentrations. Ca content in the root treated with 600  $\mu\text{g g}^{-1}$   $\text{Ca}^{2+}$  concentrations reached 34.4  $\text{g kg}^{-1}$ , which was 5.5 times as high as that of the root of the control (6.3  $\text{g kg}^{-1}$ ), and about 1.4 times as high as that of the root treated with 400  $\mu\text{g g}^{-1}$  and 200  $\mu\text{g g}^{-1}$   $\text{Ca}^{2+}$  concentrations (25.0  $\text{g kg}^{-1}$  and 24.6  $\text{g kg}^{-1}$  respectively) respectively. The changing trend of Ca content in leaf was almost the same as in root, except the difference of them among treatments with  $\text{Ca}^{2+}$  was significant. The effect of  $\text{Ca}^{2+}$  concentrations on Ca content in the bark was relatively slight, and the difference was not significant among treatments with  $\text{Ca}^{2+}$ ; however, there were significant difference between treatments and control. Multiple test on Ca content in the leaf, root and bark indicated that the difference among three components was

very significant ( $F=11.80$ ), and their content followed the order of root>leaf>bark. Ca content in root treated with 600  $\mu\text{g g}^{-1}$   $\text{Ca}^{2+}$  concentrations was 1.5 times as high as that in leaf (23.1  $\text{g kg}^{-1}$ ) and 2.3 times as high as that in the bark (15.2  $\text{g kg}^{-1}$ ) respectively. Although the effect of  $\text{Ca}^{2+}$  concentrations on Ca content in the bark was relatively slight, Ca content in the bark treated with 600  $\mu\text{g g}^{-1}$   $\text{Ca}^{2+}$  concentrations still reached 15.2  $\text{g kg}^{-1}$  and was 2.3 times as high as that of the bark of the control (6.6  $\text{g kg}^{-1}$ ), over 5.3% and 13.6% of that in the bark treated with 400 and 200  $\mu\text{g g}^{-1}$   $\text{Ca}^{2+}$  concentrations respectively.

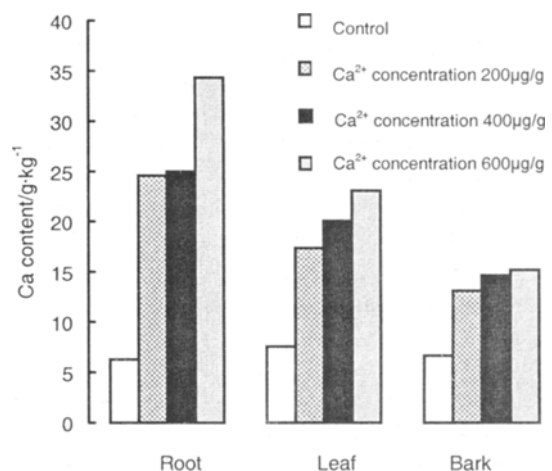


Fig. 1 The effect of  $\text{Ca}^{2+}$  concentrations on Ca contents in the root, leaf and bark

### Effects of $\text{Ca}^{2+}$ concentrations on K and Mg contents

It is possible that  $\text{Ca}^{2+}$  concentration restrains *Pteroceltis tatarinowii* to absorb K. K contents in the leaf, root and bark were much lower in treatments with  $\text{Ca}^{2+}$  than in the control (Fig. 2). Analysis of variance indicated that the difference of K contents in the three components among treatments was very significant. In the treatments with  $\text{Ca}^{2+}$ , K content in leaf treated with 200  $\mu\text{g g}^{-1}$   $\text{Ca}^{2+}$  concentrations was 45.1  $\text{g kg}^{-1}$  and significantly higher than that of the other treatments, but the difference between treatments with 400  $\mu\text{g g}^{-1}$  and 600  $\mu\text{g g}^{-1}$   $\text{Ca}^{2+}$  concentrations was not significant. Both variance analysis and Duncan's multiple range tests suggested that the difference of K contents in the root and bark was very significant between treatments with  $\text{Ca}^{2+}$  and ck (without  $\text{Ca}^{2+}$ ), but was not significant among treatments with  $\text{Ca}^{2+}$ ; moreover, K contents in the root and bark were more seriously affected by  $\text{Ca}^{2+}$  concentrations compared with the leaf.

Similar to the changing trend of K contents, Mg contents in the leaf, root and bark were also negatively related to  $\text{Ca}^{2+}$  concentrations. Compared with the treatments with  $\text{Ca}^{2+}$ , Mg contents in the leaf, root and bark of the control were the highest, for example reaching 6.6  $\text{g kg}^{-1}$  in the leaf, and the lowest in the leaf treated with 600  $\mu\text{g g}^{-1}$   $\text{Ca}^{2+}$

concentrations (Fig. 3), which was only  $5.4 \text{ g} \cdot \text{kg}^{-1}$  in leaf. Variance analysis and Duncan's multiple range tests indicated that the difference of Mg content in the root and leaf was not very significant between treatments with different  $\text{Ca}^{2+}$  concentrations, while reaching significant level in the bark.

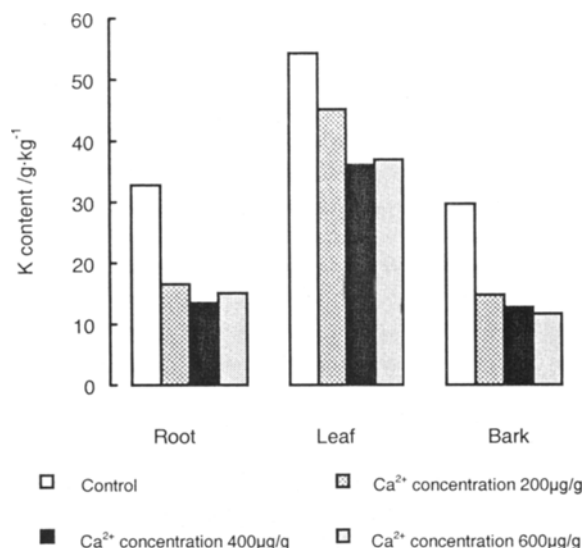


Fig. 2 The effect of  $\text{Ca}^{2+}$  concentrations on K content in the root, leaf and bark

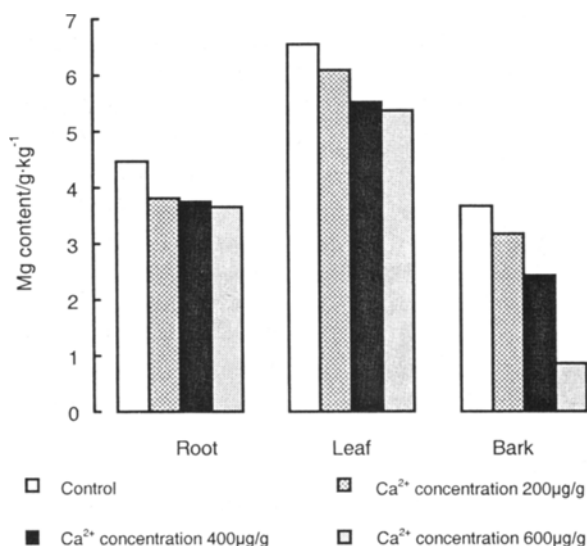


Fig. 3 The effect of  $\text{Ca}^{2+}$  concentrations on Mg

Although K and Mg contents in the bark were more seriously affected by  $\text{Ca}^{2+}$  concentrations, the distribution of them in the three components shared the same tendency: leaf>root>bark. The contents of K and Mg in the leaf treated with  $600 \text{ µg/g}$   $\text{Ca}^{2+}$  concentrations were  $36.9 \text{ g} \cdot \text{kg}^{-1}$  and  $5.4 \text{ g} \cdot \text{kg}^{-1}$  respectively, which were only 68.6% and 82.0% of that of the control respectively. K content in the bark treated with  $600 \text{ µg/g}$   $\text{Ca}^{2+}$  concentrations was 39.3% of that of the

control, and was 79.0% and 91.8% of that of the bark treated with  $200 \text{ µg/g}$  and  $400 \text{ µg} \cdot \text{g}^{-1}$   $\text{Ca}^{2+}$  concentrations respectively. Mg content in the bark treated with  $600 \text{ µg/g}$   $\text{Ca}^{2+}$  concentrations was 23.4% of that of the bark of the control, and was 27.1% and 35.4% of that of the bark treated with  $200 \text{ µg} \cdot \text{g}^{-1}$  and  $400 \text{ µg} \cdot \text{g}^{-1}$   $\text{Ca}^{2+}$  concentrations, respectively.

#### Effects of $\text{Ca}^{2+}$ concentrations on Mn, Zn, Cu and Na contents in the root, leaf and bark

Generally, Mn, Zn, Cu and Na contents in the three components were slightly affected by  $\text{Ca}^{2+}$  concentrations (Table 1). Na and Cu contents were in the order of root>bark>leaf, while Mn and Zn contents in the three components were in the order of root>leaf>bark. In the control, Mn, Zn, and Cu contents in the root were much higher than that in the other treatments, and the differences between  $\text{Ca}^{2+}$  treatments and control reached significant level ( $p=0.01$ ). Na contents in the root and bark treated with  $200 \text{ µg/g}$   $\text{Ca}^{2+}$  concentrations were higher than that in the other treatments. Mn and Zn contents in the leaf were positively related to  $\text{Ca}^{2+}$  concentrations, while Na and Cu contents were negative. The accumulation of Mn and Cu contents in the bark was slightly promoted as the  $\text{Ca}^{2+}$  concentrations increasing, while the Na accumulation in the bark was decreased.

#### Discussion

##### $\text{Ca}^{2+}$ promoting the accumulation of Ca contents in the root, bark and leaf of *Pteroceltis tatarinowii*

Ca is the required element for tree growth. Ca deficit may affect tree quality and performance. Because of Ca deficit, there were only three living seedlings in the control at last and their average height and base diameter were only 43.8 cm and 3.0 cm, respectively, indicating that Ca deficit severely restricted the growth of *Pteroceltis tatarinowii*. However, Ca accumulation ( $6.3 - 7.5 \text{ g} \cdot \text{kg}^{-1}$ ) in the components also occurred in the control treatment, which may be the result of high Ca content in *Pteroceltis tatarinowii* seeds. Ca contents in the three components in the treatment with  $\text{Ca}^{2+}$  were much higher than those in the control, and in the order of root>leaf>bark. With the increasing of  $\text{Ca}^{2+}$  concentrations, the accumulation of Ca in three components was promoted.

There was no significant difference in height and base diameter growth between the treatments with  $200 \text{ µg} \cdot \text{g}^{-1}$ ,  $400 \text{ µg} \cdot \text{g}^{-1}$  and  $600 \text{ µg} \cdot \text{g}^{-1}$   $\text{Ca}^{2+}$  concentrations. The Ca accumulation in the leaf of *Pteroceltis tatarinowii* reached  $23.1 \text{ g} \cdot \text{kg}^{-1}$ , which was greatly higher than that of common tree species, ranging from  $2 \text{ g} \cdot \text{kg}^{-1}$  to  $10 \text{ g} \cdot \text{kg}^{-1}$  (Lu 1998). It had been reported that calcium in the most tree species is not translocated internally (Waring *et al.* 1985). Therefore the Ca accumulation in the bark would be much higher with the increasing of tree age. This result showed that *Pteroceltis tatarinowii* is one kind of calcicoles species and

adapts to grow on soil with high  $\text{Ca}^{2+}$  concentrations, such as limestone soil with high carbonate. *Pteroceltis tatarinowii* bark with high Ca content can improve Xuan paper quality markedly (Liu *et al.* 1985; 1986), so additional research is

needed to test whether fertilizing appropriate Ca in non-limestone soil would promote Ca accumulation in the bark of *Pteroceltis tatarinowii*.

**Table 1. The effect of  $\text{Ca}^{2+}$  concentrations on Mn, Zn, Cu and Na content in the root, leaf and bark**

( $\mu\text{g} \cdot \text{g}^{-1}$ )

Treatments	Mineral elements											
	Mn			Zn			Cu			Na		
	Root	Leaf	Bark	Root	Leaf	Bark	Root	Leaf	Bark	Root	Leaf	Bark
Control	624.61	61.31	82.65	180.80	21.97	12.76	64.27	6.78	5.37	0.71	0.15	0.20
200 $\mu\text{g/g}$	410.05	42.57	35.00	97.65	32.89	13.90	19.97	5.86	6.63	0.86	0.21	0.51
400 $\mu\text{g/g}$	399.84	44.89	35.60	113.72	38.18	16.81	22.89	4.74	8.26	0.69	0.19	0.37
600 $\mu\text{g/g}$	362.52	63.70	41.09	106.87	42.57	12.00	27.53	7.15	7.85	0.57	0.25	0.13

### $\text{Ca}^{2+}$ concentrations restraining the accumulation of K and Mg

Some earlier experiments indicated that  $\text{Ca}^{2+}$  restricted K and Mg uptake. For example, results presented by Zhou (1995) revealed that fertilizing calcium nitrate on peanut decreased K uptake by 16.2%, suggesting that K uptake was negatively related to Ca concentrations. Our results also showed that  $\text{Ca}^{2+}$  concentrations did negative effects on K and Mg contents in the three components, especially in the bark (Fig. 2 and 3). The contents of K and Mg in the bark treated with 600  $\mu\text{g/g}$   $\text{Ca}^{2+}$  concentrations were only 39.3% and 58.9% of that of the bark of the control respectively. However, other earlier experiments also found that appropriate  $\text{Ca}^{2+}$  concentrations can promote K and Mg uptake. For example, results presented by Yang (1994) suggested that  $\text{Ca}^{2+}$  concentrations (0-160  $\mu\text{g} \cdot \text{g}^{-1}$ ) promoted the accumulation of K and Mg, while  $\text{Ca}^{2+}$  concentrations (320  $\mu\text{g} \cdot \text{g}^{-1}$ ) did inversed effects.

### Effects of $\text{Ca}^{2+}$ concentrations on the accumulation of Mn, Zn, Cu and Na contents

Mn, Cu and Zn are the trace elements for plant growth. Our experimental results showed that  $\text{Ca}^{2+}$  concentrations affected the accumulation of these elements in the root, leaf and bark. The general tendency was that increasing  $\text{Ca}^{2+}$  concentrations blocked the accumulation of Mn, Cu and Zn, and improved the Na accumulation in the root. However, the accumulation of Mn and Cu contents in the bark was slightly promoted as the  $\text{Ca}^{2+}$  concentrations increasing,

while the Na accumulation in the bark was negatively related to the  $\text{Ca}^{2+}$  concentrations. Some research results (Mengel *et al.* 1987) suggested that the availability of Mn, Cu and Zn in calcific mountainous regions is very low because there exist competitive effects between  $\text{Ca}^{2+}$  and these elements (mainly compete for absorbing positions), and high pH value in calcium soil also affects their uptake by plants. Although Na is not a necessary element for plant growth, Na had positive effects on plant growth, production and quality. Cao (1993) indicated that the mineral element content in the water was an important factor to affect quality of Xuan paper; therefore the variation of these mineral element contents in the bark would also affect the paper quality.

### The comparison of mineral elements in the bark between limestone soil and nutrient solution

Table 2 showed that Ca content in the bark of *Pteroceltis tatarinowii* growing in the solution with 600  $\mu\text{g/g}$   $\text{Ca}^{2+}$  concentrations was almost the same as growing in the limestone soil, while K, Mg, Cu and Zn contents of the bark in the solutions were much lower than that in the limestone soil, and the Mn content of the bark growing in the solutions was significantly higher than that growing in the limestone soil. The results presented by Fang *et al.* (2002), Lu (1998), Mengel *et al.* (1987) revealed that different pH values in soil had great effects on the accumulation of mineral elements in the bark.

**Table 2. The comparison between element contents in the bark of *Pteroceltis tatarinowii* growing in the limestone soil and in Hoagland nutrient solution with different  $\text{Ca}^{2+}$  concentrations**

Element	Limestone soil (pH 6.5-7.5)	Hoagland nutrient solution with different $\text{Ca}^{2+}$ concentrations/ $\mu\text{g} \cdot \text{g}^{-1}$		
		200	400	600
Total Ca ( $\text{g} \cdot \text{kg}^{-1}$ )	15.31	13.10	14.36	15.16
Total K ( $\text{g} \cdot \text{kg}^{-1}$ )	13.08	14.78	12.71	11.67
Total Mg ( $\text{g} \cdot \text{kg}^{-1}$ )	4.73	3.71	2.43	2.16
Total Mn ( $\mu\text{g} \cdot \text{g}^{-1}$ )	22.05	35.00	35.60	41.09
Total Cu ( $\mu\text{g} \cdot \text{g}^{-1}$ )	9.49	6.63	8.53	7.85
Total Zn ( $\mu\text{g} \cdot \text{g}^{-1}$ )	31.33	13.90	16.81	12.00

It is easy to find the main differences between the results from limestone soil and the results from this experiment were that the pH in the limestone soil (pH 6.5-7.5) was little higher

than that in the solution (pH 6.0-6.5), and there existed  $\text{CO}_3^{2-}$  in limestone soil while there were no  $\text{CO}_3^{2-}$  in Hoagland nutrient solution, which would affect the

availability of mineral elements and the uptake by *Pteroceltis tatarinowii*. Furthermore, these differences resulted in the variation in mineral element contents of the bark between the two trials.

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